REMARKS/ARGUMENTS

Before turning to the status of claims and the rejections, Applicants, again, request that the Examiner note the new Power of Attorney and Change of Address form filed with Applicants' prior response on February 9, 2006. Unfortunately, despite the change in correspondence address, the Office Action of June 12, 2007 was still mailed to Applicants. The Examiner is respectfully requested to update the records, and mail all future communications to the current address, to the attention of the undersigned attorney.

Claims 1-13 and 15-25 are pending in this application and stand rejected under various grounds. Claim 1 has been amended. The amendment of claim 1 is of formal nature and does not add new matter. The amendment was made without prejudice or disclaimer. Applicants explicitly retain the right to pursue any deleted subject matter in one or more continuing applications.

Rejections Withdrawn

Applicants note and appreciate withdrawal of the rejections of clams 1-13 and 15-25 under 35 U.S.C. 103(a) stated in the previous Office Actions.

New Claim Rejections - 35 U.S.C. §103

(1) Claims 1-13 and 15-24 have been rejected under 35 U.S.C. 102(a) as allegedly being unpatentable over Hart et al. (BIO/TECHNOLOGY Col. 12, November 1994) in view of the combined teachings of Wetzel et al. (EP 0155189) and Van Dien et al. (Appl Environ Microbiol. 1997, 63(5):1689-95).

The Rejection

Hart et al. (especially pp. 1113-115) was cited for its teaching of a process for large-scale production of IGF-I from the periplasm of *E. coli* by culturing *E. coli* host cells having a plasmid comprising an inducible alkaline phosphatase promoter and nucleic acid encoding a human IGF-I linked to a *lamb* signal sequence for secretion into the periplasm.

Wetzel et al. (especially pp. 3-7 and claims 1-9) was cited for teaching a plasmid vector comprising an inducible promoter and nucleic acid encoding a T4 phage lysozyme.

Van Dien et al. (especially Results and Discussion and pp. 1689-1693) was cited for its alleged teaching of genes involved in polyphosphate metabolism in *E. coli* that were cloned behind different inducible promoters on separate plasmids.

According to the rejection, it "would have been obvious to one of ordinary skill in the art at the time the invention was made to place the nucleic acid encoding a T4 phage lysozyme taught by Wetzel et al. behind the arabinose inducible P_{BAD} promoter and/or place the nucleic acid encoding a human IGF-I linked to a *lamb* signal sequence for secretion into the periplasm taught by Hart et al. behind the IPTG inducible P_{tac} promoter," and to "further transform the *E. coli* cells of Hart et al. with the modified plasmid vectors of Wetzel et al. and/or the modified plasmid vector having the nucleic acid encoding a human IGF-I linked to a *lamb* signal sequence placed behind the IPTG inducible P_{tac} promoter." (Office Action, page 3) The Examiner adds that the additional steps recited in claim 1 would have been obvious to one of ordinary skill in the art at the time the invention was made.

The Examiner finds motivation to make the combination "in order to have synthesis of lysozyme that ruptures the polysaccharide membrane of *E. coli* host cell after accumulation of human IGF-I in the periplasm which simplified the purification of the human IGF-I." (Office Action, page 3)

A prima facie case of obviousness has not been established

Applicants submit that a *prima facie* case of obviousness has not been established and respectfully traverse the rejection.

The process claimed in claim 1 involves the recovery of refractile particles containing a heterologous polypeptide from bacterial periplasm in which the polypeptide is insoluble, using bacterial cells, which comprise (i) nucleic acid encoding a desired heterologous polypeptide including a secretory signal sequence under control of a first inducible promoter; and (ii) nucleic acid encoding a phage lysozyme under control of a second, different, inducible promoter. First, the production of the desired heterologous polypeptide is initiated by inducing the first inducible promoter, resulting in the secretion of the heterologous polypeptide into the periplasm of the bacteria as an aggregate. Once 50% or more of the heterologous polypeptide has accumulated, production of the phage lysozyme is initiated, by inducing the second inducible promoter, resulting in the accumulation of the phage lysozyme in the cytoplasmic compartment of the

bacteria. As a result of this coordinated expression schedule, the insoluble refractile bodies are efficiently released from the entanglement in the peptidoglycan layer, resulting in a significant increase in the amount of insoluble heterologous polypeptide that can be recovered from the periplasm of the bacteria by subsequent mechanical disruption of the bacterial cells.

Applicants do not contest that, as evidenced by the cited combination of Hart et al., Wetzel et al. and Van Dien et al, inducible promoters, vectors containing the coding sequences of heterologous polypeptides under control of inducible promoters, vectors containing the coding sequence of a lysozyme under control of an inducible promoter, and bacterial cells transfected with two separate expression vectors, each containing a different coding sequence under control of a different inducible promoter were known in the art at the time the present invention was made.

However, the invention claimed in the present application goes way beyond this knowledge. As explained in the specification, and as it is clearly reflected in the claims, the process disclosed and claimed in the present application requires the *coordinated expression* of nucleic acid encoding a desired heterologous polypeptide and nucleic acid encoding a phage lysozyme, in which *expression of the phage lysozyme is induced only after about 50% or more of the heterologous polypeptide has accumulated.*

There is nothing in the cited reference, when taken alone or in any combination, suggesting the coordinated expression of a nucleic acid encoding a heterologous protein and a nucleic acid encoding a phage lysozyme, where expression of the latter is induced only after about 50% or more of the heterologous polypeptide has accumulated. Accordingly, the cited combination of references does not result in the invention claimed in the present application and thus does not make obvious the claimed invention.

In the only statement that concerns the coordinated expression of the two nucleic acid molecules, the Examiner asserts that "[o]ne of ordinary skill in the art at the time the invention was made would have been motivated to wait until 50% or more of the human IGF-I has accumulated before inducing with arabinose to express T4 phage lysozyme in order to obtain a greater yield of human IGF-I." (Office Action, page 3) The Examiner fails to provide any evidence in support of this assertion. Indeed, as explained at page 9, lines 29-32 of the specification:

"...it would not be expected that induction at the end of a long fermentation process and after substantial product accumulation would produce enough of the phage lysozyme to be effective."

In *Graham v. John Deere*, 383 U.S. 1, 36, the Supreme Court warned against "the temptation to read into prior art the teachings of the invention in issue" and instructed the courts "to guard against slipping into the use of hindsight." It appears that it is exactly this legally improper hindsight reasoning that the Examiner has engaged in, using the teaching of the present application, and nothing else, to supply a key element of the claimed invention.

In conclusion, the cited combination of references fails to teach a key element of the present invention, and therefore the present rejection is misplaced and should be withdrawn.

(2) Claim 25 has been rejected under 35 U.S.C. 103(a) over the same combination of references as that cited in the rejection above, and further in view of Balbas et al., Gene 1996, 172(1):65-9. Balbas et al. was cited for teaching the plasmid pBRINT which is characterized as being "an efficient vector for chromosomal integration of cloned DNA into the lacZ gene of *Escherichia coli*." According to the rejection, it would have been obvious to combine the teaching of this reference with the other references cited and such combination would result in teaching the integration of a nucleic acid into the chromosome of a bacterial cell, as claimed in claim 25.

In response to the previous rejection, Applicants have shown that the cited combination of the primary references does not make obvious the invention claimed in claims 1 -13 and 15-24. Since claim 25 depends from claim 1, and Balbas et al. does not provide the teaching missing from the first three references, claim 25 is not obvious either, and the present rejection should be withdrawn.

Claim Rejections – 35 U.S.C. §112, 2nd Paragraph

Claims 1-13 and 15-25 have been rejected under 35 U.S.C. 112, second paragraph for alleged indefiniteness. According to the rejection, the phrase "under conditions whereby the heterologous polypeptide is secreted into the periplasm of the bacteria as an aggregate and the phage lysozyme accumulates in the cytoplasmic compartment" renders claim 1 vague and

indefinite since such specific conditions "are not defined and described in the specification" and vary from one practitioner to another in the art of recombinant production technology."

Without acquiescing to the rejection, or the Examiner's reasoning in support of the rejection, claim 1 has been amended to remove a reference to any specific conditions. Accordingly, the present rejection is believed to be moot. It is noted that the current claim language includes all steps necessary to achieve the desired result, and the secretion of the heterologous polypeptide into the periplasm of the bacteria as an aggregate and the accumulation of the phage lysozyme in the cytoplasmic compartment of the bacteria are a natural result of the expression of the polypeptide and lysozyme, respectively, as recited in the claim. Accordingly, the recitation of the deleted expression was unnecessary.

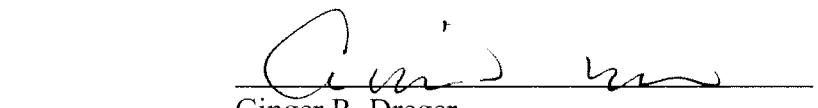
All claims pending in this application are believed to be in *prima facie* condition for allowance, and an early action to that effect is respectfully solicited.

Please charge any additional fees, including any additional fees for extension of time, or credit overpayment to Deposit Account No. **08-1641** (Attorney's Docket No. **39766-0128 A**).

Please direct any calls in connection with this application to the undersigned at the number provided below.

Respectfully submitted,

Date: December 12, 2007



Ginger R. Dreger
Reg. No. 33,055

HELLER EHRMAN LLP
275 Middlefield Road
Menlo Park, California 94025
Telephone: (650) 324-7000
Facsimile: (650) 324-0638